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Gender difference in the glucagon response to glucopenic stress in mice

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Karlsson, Sven, Anton J. W. Scheurink, and Bo Ahrén. Gender difference in the glucagon response to glucopenic stress in mice. *Am J Physiol Regulatory Integrative Comp Physiol* 282: R281–R288, 2002.—A gender difference in the glucagon response to insulin-induced hypoglycemia was previously demonstrated in humans. Whether this reflects a gender difference in autonomic activation or in pancreatic α-cell regulation is not known. We investigated the glucagon, epinephrine, and norepinephrine responses to neuroglycopenic stress induced by 2-deoxy-D-glucose (2-DG) or insulin in female and male mice. 2-DG increased plasma glucagon levels by 559 ± 68% in females versus 281 ± 46% in males ($P < 0.01$). Plasma levels of epinephrine or norepinephrine after 2-DG administration did not differ between genders. During insulin-induced hypoglycemia, the glucagon response was similarly higher in females ($P < 0.001$), whereas the plasma catecholamine response was higher in males ($P < 0.05$). In vivo, the glucagon response to carbachol or clonidine was higher in females ($P < 0.05$). In isolated islets, the glucagon response to carbachol (100 μM; $P = 0.003$) but not to clonidine (1 μM) was larger in females. We conclude that in addition to a larger α-cell mass (previously described in female mice), an increased sensitivity of the glucagon-producing α-cell to cholinergic activation contributes to the larger glucagon response to glucopenic stress in female mice.

hypoglycemia; autonomic nervous system; epinephrine; norepinephrine; males; females

THE EARLY COUNTERREGULATORY RESPONSE to insulin-induced hypoglycemia involves autonomic activation and increased glucagon secretion to restore plasma glucose levels (4, 8, 13, 15, 31). Several studies on the counterregulatory glucagon and catecholamine responses to insulin-induced hypoglycemia reported an intriguing gender difference in healthy human subjects as well as in diabetic subjects (3, 9, 11). Thus although all of these studies revealed that the epinephrine response to insulin-induced hypoglycemia was lower in females than in males, a diminished glucagon response in females was reported in some (9, 11) but not all (3) studies.

It is not known whether the gender difference in the autonomic response to insulin-induced hypoglycemia underlies the difference in glucagon secretion. In addition, although many studies regarding glucopenic stress have been performed in rodents and other species (for a review, see Ref. 15), data regarding gender differences in the counterregulatory response to glucopenic stress are scarce for species other than humans. The aim of the present study was first to explore whether a gender difference in the autonomic and glucagon-secretion responses to glucopenic stress does exist in mice, and if so, whether these differences are caused by changes in autonomic activation or by changes in α-cell activity. Both insulin-induced hypoglycemia and the glucose analog 2-deoxy-D-glucose (2-DG) were used in this study as models for glucopenic stress.

The glucose analog 2-DG activates the autonomic nervous system through central neuroglycopenia (7, 15, 24, 32) and allows us to dissociate the effects of hypoglycemia from those of insulin. Thus interpretation of results using insulin-induced hypoglycemia to induce glucopenic stress is hampered by findings that high concentrations of insulin per se may exaggerate the sympathetic autonomic response to insulin-induced hypoglycemia in normal human subjects (9, 10). Moreover, exogenous insulin itself may inhibit glucagon secretion as well as endogenous insulin secretion (27, 31).

In the present study, the following two experiments were performed on mice: *1*) comparison of the early glucagon and plasma catecholamine responses to 2-DG-induced neuroglycopenia or insulin-induced hypoglycemia in female and male mice, and *2*) comparison of the glucagon-producing α-cell response to direct cholinergic or adrenergic activation between genders both in vivo and in vitro in isolated islets of Langerhans.

MATERIALS AND METHODS

Animals. Nonfasted, unanesthetized male (body wt 35–45 g) and female (body wt 25–35 g) mice (age 12–14 wk) of the NMRI strain (Bolmholdtgaard Breeding and Research Center, Ry, Denmark) were used throughout the study. The animals were fed a standard pellet diet and tap water ad libitum. Experiments were performed between 1 and 3 PM. The time of the estrous cycle of the female mice was not considered. The study was approved by the Ethics Committee for Animal Research of Lund University.

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Studies in vivo. We injected 2-DG (Sigma Chemical, St. Louis, MO) at a dose of 0.3, 1.5, or 3 mmol/kg iv into a tail vein (volume load 10 μl/kg body wt). Control animals were injected with 0.9% NaCl. Blood was sampled from the retrobulbar plexus after 2 or 10 min for the determination of glucose, insulin, and glucagon values. In separate experiments, blood was sampled at 2 or 10 min after injection of 2-DG (3 mmol/kg iv) for determination of plasma levels of epinephrine and norepinephrine. For these time points, the insulin and glucagon responses to 2-DG (3 mmol/kg) have previously been shown to be markedly increased in female mice (16, 18). Insulin-induced hypoglycemia in mice was performed by injection of insulin (2 U/kg body wt ip; Actrapid, Novo Nordisk, Bagsvaerd, Denmark) as previously described (14). Controls were injected with 0.9% NaCl. Blood was sampled 15 min after the intraperitoneal injection. Plasma was separated by centrifugation, and samples were stored at −20°C until analysis for insulin, glucagon, and glucose contents. Blood samples (250 μl) for catecholamine determination were immediately transferred into chilled tubes containing 10 μl of heparin (500 IU/ml; Lövens Chemical Industries, Ballerup, Denmark) and EDTA (10 mg/ml heparin; Merck, Darmstadt, Germany) and centrifuged, and plasma was stored at −70°C until assay for catecholamine content. To study the acute glucagon response to cholinergic or adrenergic stimulation, male and female mice were injected (volume load 10 μl/kg body wt) with the muscarinic agonist carbachol (0.16 μmol/kg iv) or the α_2-adrenergic agonist clonidine (50 nmol/kg iv), and blood was sampled after 2 min. At these dosage levels, carbachol or clonidine, respectively, have previously been shown to induce a peak increase in the plasma glucagon levels 2 min after injection in female mice (2, 29).

Isolation and incubation of islets. Islets from male and female mice were isolated by the collagenase-digestion technique as initially described by Lacy and Kostianovsky (19) with slight modifications. In short, during pentobarbital sodium (120 mg/kg) anesthesia, a catheter was inserted through the common bile duct and the duodenal papilla was occluded. Thereafter the pancreas was retrogradely filled with 3 ml of Hanks' balanced salt solution (Sigma) supplemented with 0.3 mg/ml of collagenase (Collagenase P, Boehringer Mannheim, Germany). The pancreas was removed and incubated at 37°C for 20–22 min. Islets were thereafter hand-picked under a stereomicroscope and cultured overnight in RPMI-1640 medium (GIBCO, Paisley, UK) supplemented with 10% fetal calf serum (Biological Industries, Kibbutz Beit Haemek, Israel), 2.06 mmol/l L-glutamine, 100 IU/ml penicillin, 100 μg/ml streptomycin (Biological Industries), and 2.5 μg/ml amphotericin B (GIBCO). After the overnight culture period, the islets were preincubated for 60 min in HEPES buffer supplemented by 0.1% human serum albumin and 3.3 mM glucose. The buffer contained (in mM) 25 HEPES, 125 NaCl, 5.9 KCl, 1.28 $CaCl_2$, and 1.1 $MgCl_2$. The pH (7.36) of the HEPES buffer was adjusted with NaOH. Batches of three islets were thereafter incubated in a 200-μl volume of HEPES buffer supplemented with human serum albumin, 1.8 mM glucose, and 5 mM L-arginine (Sigma) for 60 min at 37°C in an atmosphere of humidified air saturated with 5% CO_2. During the experiments, 0.1 mM carbachol (Sigma), 1 μM clonidine (Sigma), or 10 mM L-arginine was added according to the protocols. After the incubation period, medium was removed and analyzed by radioimmunoassay for insulin (25 μl) and glucagon (100 μl) contents. Aprotinin (Trasylol; Bayer, Leverkusen, Germany) at a final concentration of 1,000 kIU/ml was added to the test tubes for glucagon analysis.

Determinations of insulin, glucagon, glucose, and catecholamines. Insulin was determined by radioimmunoassay using a guinea pig anti-rat insulin antibody (^{125}I-labeled human insulin) as a tracer and rat insulin as a standard (Linco Research, St. Charles, MI). The separation of free and bound radioactivity was performed via the double-antibody technique using a goat anti-guinea pig IgG antibody (Linco). Plasma glucagon levels were determined by radioimmunoassay using the glucagon antibody ^{125}I-glucagon and a glucagon standard from Linco. Plasma glucose levels were determined with the glucose oxidase method. Epinephrine and norepinephrine were analyzed from plasma by liquid chromatography in combination with electrochemical detection as previously described (26, 28).

Statistics. Data are presented as means ± SE. One-way ANOVA preceded the Bonferroni test or the Student-Newman-Keuls test for correction of multiple comparisons or Student's *t*-test for unpaired observations was used for statistical evaluation. A value of $P < 0.05$ was considered significant. When comparisons were made between males and females with respect to any differences in magnitude of the response to a certain agonist, the differences in plasma levels of insulin or glucagon compared with NaCl-injected controls were also calculated and expressed in either absolute figures or in percentages (controls set to 100%). For evaluation of any differences between females and males in plasma levels of insulin, glucagon, and glucose during control conditions, all NaCl-injected control animals from which blood was sampled after 2 min were included for statistical comparison.

RESULTS

Baseline plasma glucagon, insulin, and glucose levels. Values of plasma insulin, glucagon, and glucose levels obtained from all NaCl-injected animals where blood was sampled after 2 min are summarized in Table 1. Males exhibited higher plasma insulin and lower plasma glucagon levels compared with females under these conditions.

Glucagon and insulin response to 2-DG-induced neuroglycopenia. At 10 min after injection of 2-DG (1.5 or 3.0 mmol/kg iv), plasma levels of glucagon, insulin, and glucose markedly increased in both male and female mice (Fig. 1). In contrast, at 0.3 mmol/kg of 2-DG, no effect on these parameters was observed ($n = 16$; not significant; data not shown). The glucagon response to 2-DG (1.5 or 3.0 mmol/kg) was, however, markedly higher in females compared with males ($P < 0.001$; Fig. 1*A*). Thus at the 1.5 mmol/kg dose of 2-DG, plasma glucagon levels increased by 559 ± 68% in females but only 281 ± 46% in males ($P < 0.01$; $n = 10$/group). Also

Table 1. *Basal plasma levels of insulin, glucagon, and glucose in male and female mice*

	Male	Female	*P* Value
P-insulin, pmol/l	420 ± 43	238 ± 27	0.001
P-glucagon, pg/ml	98 ± 11	195 ± 29	0.003
P-glucose, mmol/l	9.3 ± 0.3	9 ± 0.2	0.377

Values are means ± SE. Plasma levels of insulin (P-insulin), glucagon (P-glucagon), and glucose (P-glucose) levels in nonfasted female ($n = 36$) and male ($n = 34$) mice at 2 min after intravenous injection of NaCl. Animals were used as controls in experiments presented in Figs. 3 and 4.

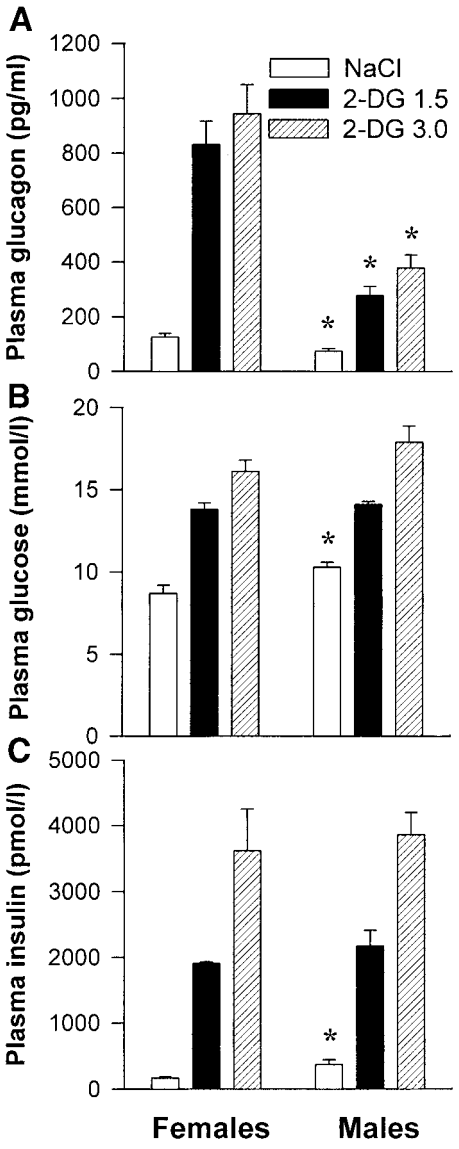

Fig. 1. Plasma levels of glucagon (*A*), glucose (*B*), and insulin (*C*) 10 min after an injection of 2-deoxy-D-glucose (2-DG) at concentrations of 1.5 or 3.0 mmol/kg iv in female and male mice. Controls were injected with 0.9% NaCl. Means ± SE of 10 animals in each group are shown; $^*P < 0.05$ between males and females.

at the earlier time point of 2 min, plasma glucagon levels were markedly higher in females compared with males in response to 2-DG (Table 2). In contrast, plasma levels of insulin after 2-DG were increased to the same extent in both genders (Fig. 1*C*). The glucose response was the same for the two doses of 2-DG; however, a higher plasma insulin level was obtained after injection of 3.0 mmol/kg compared with 1.5 mmol/kg of 2-DG in both females ($P = 0.01$) and males ($P = 0.004$). Plasma glucose levels after NaCl injection were slightly higher in males compared with females (10.2 ± 0.4 vs. 8.7 ± 0.5 mmol/l; $P = 0.011$) but did not differ between genders after injection of 2-DG at a dose of 1.5 mmol/kg ($P = 0.378$) or 3.0 mmol/kg ($P = 0.157$). Furthermore, NaCl-injected male controls exhibited higher plasma levels of insulin (369 ± 69 vs. 166 ± 18 pmol/l; $P = 0.011$) and lower plasma levels of glucagon (74 ± 10 vs. 126 ± 13 pg/ml; $P = 0.004$) compared with NaCl-injected female controls. These results show that the glucagon but not the insulin or glucose responses to 2-DG-induced neuroglycopenia are markedly higher in female compared with male mice.

Sympathoadrenal activation by 2-DG. To investigate whether the gender difference in the glucagon response to 2-DG is caused by gender differences in the activation of the sympathetic branch of the autonomic nervous system, plasma epinephrine and norepinephrine were measured 10 min after 2-DG-injection in female and male mice. It was found that 2-DG (3.0 mmol/kg) administration leads to a significant increase in plasma epinephrine levels ($P < 0.001$ for both females and males) but not norepinephrine levels (Fig. 2). There was no gender difference in the epinephrine response. The only significant gender difference observed was in norepinephrine levels after NaCl injection that were slightly higher in males than in females (5.7 ± 0.7 vs. 3.3 ± 0.4 ng/ml; $P = 0.028$). Also at the earlier time point of 2 min, there were no differences in the epinephrine or norepinephrine levels after 2-DG administration between genders, although plasma norepinephrine levels were slightly higher after NaCl injection in males compared with females ($P < 0.05$; Table 2). At this time point, 2-DG did not induce any significant change in either epinephrine or norepinephrine levels (Table 2). These results show that the degree of sympathoadrenal activation after 2-DG-induced neuroglycopenia, as reflected by plasma catecholamine levels, is similar in male and female mice.

Acute glucagon response to cholinergic or adrenergic activation. We next aimed to investigate whether the gender difference in glucagon response to 2-DG-induced neuroglycopenia may be attributed to a difference in the sensitivity of the islet glucagon-producing cells to cholinergic or α_2-adrenergic stimulation. Mice were injected with the muscarinic agonist carbachol or

Table 2. *Plasma levels of glucagon, epinephrine, and norepinephrine at 2 min after 2-deoxy-D-glucose injection*

	Female		Male	
	NaCl, 0.9%	2-DG (3 mmol/kg)	NaCl, 0.9%	2-DG (3 mmol/kg)
Glucagon, pg/ml	111 ± 14	836 ± 51	105 ± 6	242 ± 23*
Epinephrine, ng/ml	6.3 ± 0.7	8.3 ± 0.4	7.2 ± 0.7	8.6 ± 0.6
Norepinephrine, ng/ml	7.2 ± 0.8	6 ± 0.5	10.4 ± 0.9*	8.2 ± 1

Values are means ± SE of measurements on plasma from 10 mice in each group. Experiments were performed on two different occasions with 5 mice in each group. $^*P < 0.05$, significant difference between males and females. 2-DG, 2-deoxy-D-glucose.

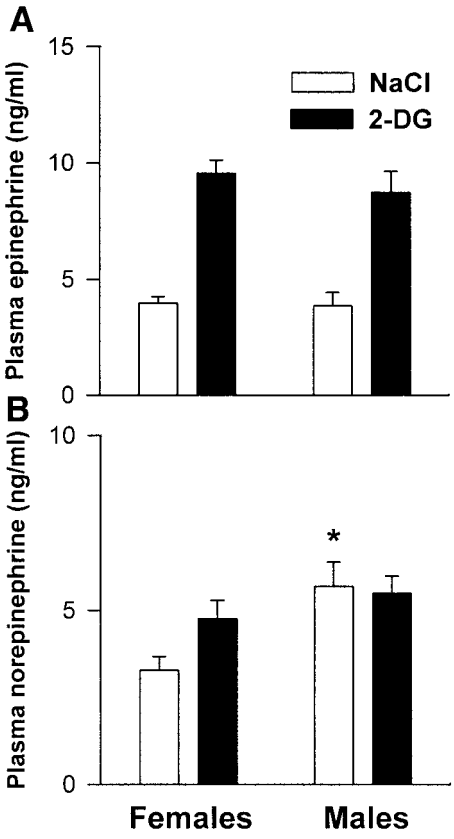

Fig. 2. Plasma levels of epinephrine (*A*) and norepinephrine (*B*) in female and male mice 10 min after an injection of 2-DG (3.0 mmol/kg iv). Controls were injected with NaCl. Means ± SE of 10 animals in each group are shown; *$P < 0.05$ between males and females.

the α_2-adrenergic agonist clonidine. These drugs stimulate glucagon secretion through direct action on the α-cells. Carbachol (0.16 μmol/kg) increased plasma glucagon levels by 402 ± 79 pg/ml in females and 90 ± 13 pg/ml in males (Fig. 3*A*; $P = 0.001$ males vs. females), which corresponds to a 140 ± 30% increase in females and a 71 ± 16% increase in males ($P = 0.044$). Plasma insulin levels after carbachol injection did not differ between genders ($P = 0.572$). However, because plasma levels of insulin were higher in males during control conditions, the calculated increase in insulin after carbachol injection was lower in males (increase of 637 ± 116 in males vs. 1,174 ± 226 pmol/l in females; $P = 0.042$). Plasma glucose levels were slightly higher in carbachol-injected males compared with females (11.1 ± 0.4 vs. 9.9 ± 0.2 mmol/l; $P = 0.014$). The adrenergic agonist clonidine (50 nmol/kg) increased plasma glucagon levels from 81 ± 6 to 232 ± 16 pg/ml in females ($P < 0.001$) and from 54 ± 8 to 97 ± 10 pg/ml in males ($P = 0.054$; Fig. 4*A*). Thus clonidine increased plasma glucagon levels by 189 ± 20% in females and 79 ± 19% in males ($P < 0.001$, male vs. female). Plasma glucose levels were slightly elevated by clonidine in males ($P = 0.003$) but were not affected in females. Plasma insulin levels did not differ between groups. In summary, these results show that the acute glucagon response to cholinergic or adrenergic activation is markedly higher in female mice compared with males.

Glucagon secretion from isolated islets. To directly study any differences in the sensitivity of the glucagon-producing α-cells to autonomic activation, isolated islets were used. The basal medium contained 1.8 mM glucose and 5 mM L-arginine to optimize the conditions for stimulation of glucagon secretion. The muscarinic receptor agonist carbachol (100 μM) stimulated glucagon secretion to a greater extent in islets from female mice compared with those from males (10.6 ± 1.1 pg·islet^{-1}·60 min^{-1} in females vs. 5.8 ± 0.9 pg·islet^{-1}·60 min^{-1} in males; $P = 0.003$; $n = 16$ in each group; Fig. 5*A*). Glucagon secretion was increased by 10 mM L-arginine or clonidine (5 μM) to a similar extent in islets from both genders (Fig. 5*A*). Insulin secretion

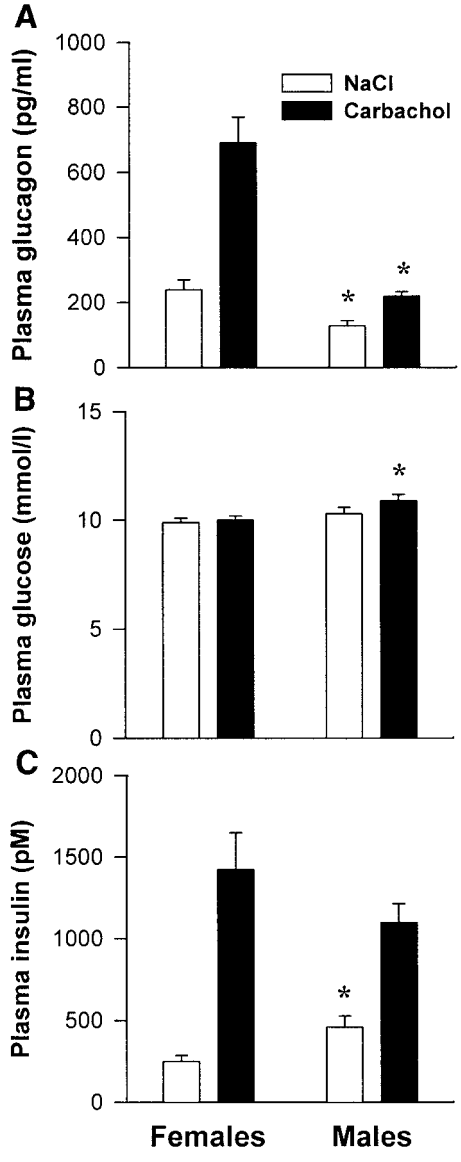

Fig. 3. Plasma levels of glucagon (*A*), glucose (*B*), and insulin (*C*) 2 min after injection of the muscarinic agonist carbachol (0.16 μmol/kg iv). Controls were injected with NaCl. Means ± SE of 20 animals in each group are shown; *$P < 0.05$ between males and females.

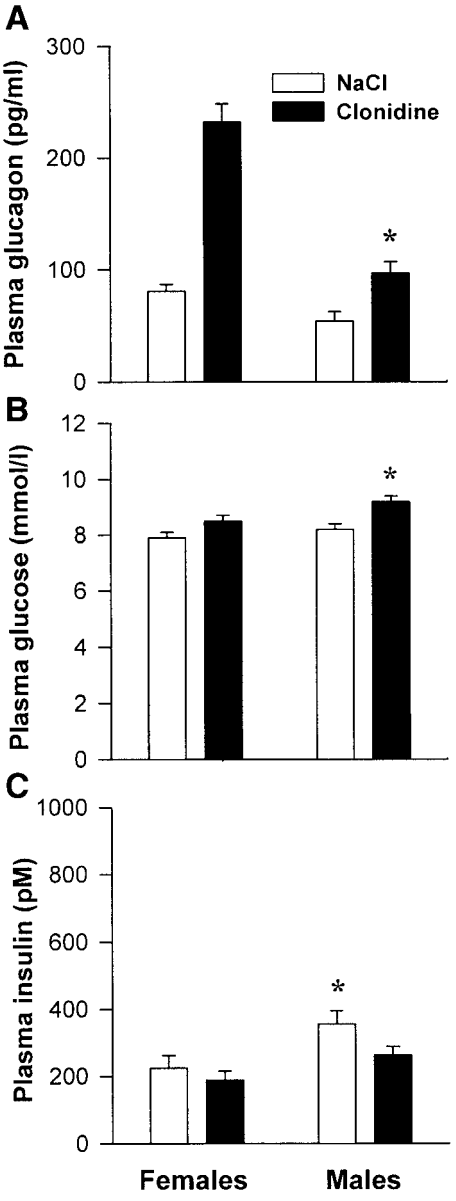

Fig. 4. Plasma levels of glucagon (*A*), glucose (*B*), and insulin (*C*) 2 min after an injection of the α_2-adrenergic agonist clonidine (50 nmol/kg iv). Controls were injected with NaCl. Means ± SE of 15–16 animals in each group are shown; *$P < 0.05$ between males and females.

monitored from the same experiments revealed an increased insulin secretory response to carbachol in islets from females compared with those from males ($P < 0.001$; Fig. 5*B*). These experiments therefore reveal an increased sensitivity of the glucagon-producing α-cells of female mice to cholinergic stimulation.

Insulin-induced hypoglycemia. Because our results of an increased glucagon response to 2-DG-induced glucopenic stress in female mice is in contrast to previous studies in humans using insulin-induced hypoglycemia to induce glucopenic stress, insulin-induced hypoglycemia was also investigated in mice. At 15 min after injection of insulin (2 U/kg ip), plasma glucose levels were 3.5 ± 0.1 mmol/l in females and 3.8 ± 0.1 mmol/l in males (not significant; Fig. 6). In NaCl-injected controls, plasma glucagon levels were 188 ± 29 pg/ml in females and 64 ± 5 pg/ml in males ($P < 0.001$). Plasma glucagon levels were elevated to 1,069 ± 88 in females and 307 ± 39 in males ($P < 0.001$). Thus after insulin-induced hypoglycemia, plasma glucagon levels are considerably higher in female compared with male mice.

Sympathoadrenal activation by insulin-induced hypoglycemia. In a separate series of experiments, the plasma epinephrine and norepinephrine responses to insulin-induced hypoglycemia were explored. It was found that both plasma epinephrine and norepinephrine levels were higher in males compared with females 15 min after insulin injection ($P < 0.05$; Table 3). There were no differences in the degree of glycemia between genders (4.2 ± 0.4 mmol/l in females vs. 4.4 ± 0.4 mmol/l in males; not significant). Also in this series of experiments, the glucagon response to insulin-induced hypoglycemia was considerably higher in females compared with males ($P < 0.001$; Table 3). Thus despite the lower plasma catecholamine response, a higher plasma glucagon response is observed in females after insulin-induced hypoglycemia, which sug-

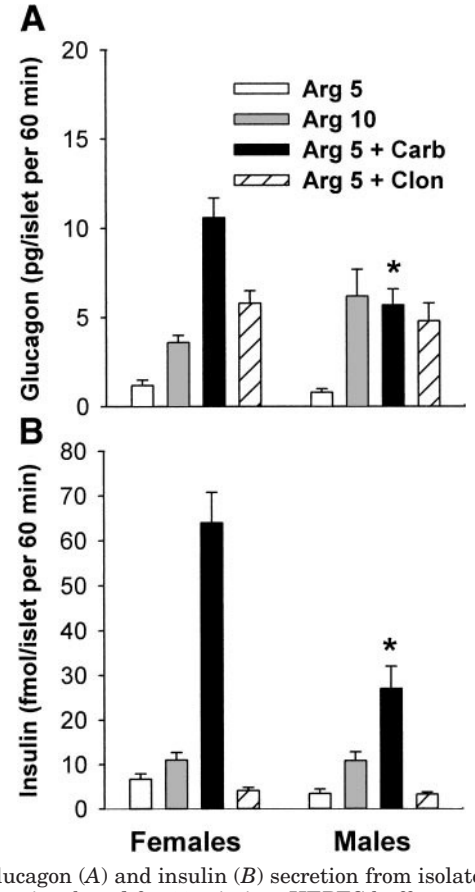

Fig. 5. Glucagon (*A*) and insulin (*B*) secretion from isolated islets of Langerhans incubated for 60 min in a HEPES buffer containing 1.8 mM glucose with the addition of either 5 or 10 mM L-arginine (Arg 5 and Arg 10, respectively), 5 mM L-arginine + 100 μM carbachol (Arg 5 + Carb), or 5 mM L-arginine + 1 μM clonidine (Arg 5 + Clon). Means ± SE of 14–16 batches of 3 islets/batch are shown; *$P < 0.05$, islets from males vs. females.

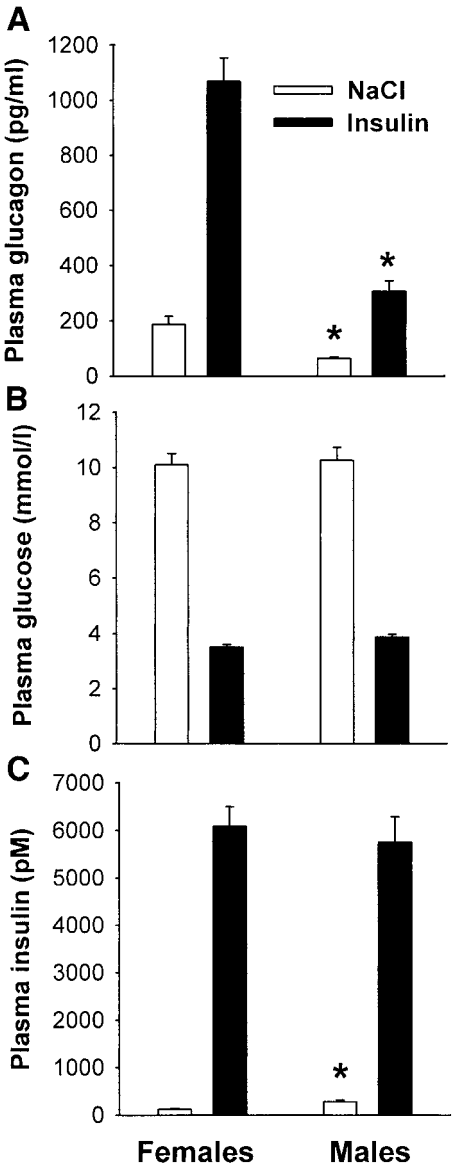

Fig. 6. Plasma levels of glucagon (*A*), glucose (*B*), and insulin (*C*) at 15 min after injection of insulin (2 U/kg body wt ip) or NaCl. Means ± SE of 18–20 animals/group are shown; $*P < 0.05$ between males and females.

gests that the mechanism underlying the higher glucagon response to glucopenic stress in female mice does not rely on differences in the degree of sympathoadrenal activation.

DISCUSSION

The main finding from the present study is that female mice have a larger glucagon response to 2-DG and insulin-induced hypoglycemia than their male counterparts, and that this increased response may be attributed to increased sensitivity of the glucagon-producing α-cells to cholinergic activation rather than to increased sympathoadrenal activation.

It has been shown that 2-DG induces autonomic nervous activation and increases glucagon secretion in several species including humans (15). It has also been demonstrated that in female mice, both the 2-DG- and hypoglycemia-induced increases in glucagon secretion are caused by simultaneous activation of the cholinergic and adrenergic branches of the autonomic nervous system (14, 16–18). Hence ganglionic, muscarinic, as well as α-adrenergic blockade inhibit the glucagon response. In the present study, we demonstrated a markedly higher glucagon response to 2-DG or insulin-induced hypoglycemia in female compared with male mice. This difference could not be attributed to gender differences in the degree of sympathoadrenal activation, because the plasma epinephrine or norepinephrine levels did not differ between males and females after 2-DG-injection, and those levels were higher in males compared with females during insulin-induced hypoglycemia. Instead, our study suggests that the difference between genders in the glucagon response relies on a higher glucagon secretory response to the given degree of autonomic activation in females, because both carbachol- and clonidine-induced glucagon secretion were enhanced in females compared with males. It has previously been shown that the glucagon pancreatic content is increased in female compared with male mice (5, 6). The possibility therefore exists that an increased α-cell mass might contribute to the larger glucagon response observed in females. The experiments in isolated islets, however, revealed that the glucagon response to cholinergic but not adrenergic activation was enhanced in islets from females compared with males. This implies that in addition to an increased α-cell mass in females, an increased sensitivity of the glucagon-producing α-cell to cholinergic activation exists in females. Theoretically, a gender difference in the degree of parasympathetic activation by glucopenic stress might also underlie the gender difference in the glucagon response. Such a difference cannot be excluded by the present study, but even if

Table 3. *Plasma levels of glucagon, epinephrine, and norepinephrine during insulin-induced hypoglycemia*

	Female		Male	
	NaCl, 0.9%	Insulin (2 U/kg)	NaCl, 0.9%	Insulin (2 U/kg)
Glucagon, pg/ml	110 ± 15	495 ± 66	87 ± 6	196 ± 23*
Epinephrine, ng/ml	6.1 ± 1.2	8.4 ± 0.4	7.1 ± 0.8	11.2 ± 1.1*
Norepinephrine, ng/ml	6.2 ± 0.9	6.7 ± 0.5	9.2 ± 1.2	13.1 ± 1.7*

Values are means ± SE. Plasma levels of glucagon, epinephrine, and norepinephrine 15 min after intraperitoneal injection of insulin. Values are measurements of plasma ($n = 10$ mice/group). Experiments were performed on two different occasions ($n = 5$ mice/group). $*P < 0.05$, significant difference between males and females.

such a difference exists, our study demonstrates that an increased sensitivity to cholinergic activation might contribute to the gender difference in the glucagon response to glucopenic stress in mice. This increased sensitivity could theoretically be attributed to gender differences in the number or the functioning of cholinergic receptors on the pancreatic α-cells under the influence of, for example, sex hormones. Whether any such influences exist with respect to the α-cell is not known. On the contrary, it has been shown previously (12) that testosterone or progesterone (in contrast to corticosteroids) does not affect α_2-adrenoceptor density in insulin-producing HIT-T15 cells.

An intriguing observation of the present study was that the glucagon response to the α_2-adrenoceptor agonist clonidine was higher in females under in vivo conditions but not in isolated islets. This points to a gender difference in the sensitivity to the central action, i.e., an indirect, extra-islet action of clonidine. Clonidine is known to decrease central sympathetic outflow by inhibiting hypothalamic norepinephrine release by presynaptic action in combination with hyperglycemia induced by a central postsynaptic mechanism (22, 30). Two possible mechanisms might therefore explain our results: *1*) clonidine induces a more pronounced decrease in sympathetic outflow from the central nervous system in males compared with females concomitantly with an equal effect on the postsynaptic receptors on the glucagon cells; and *2*) the increase in plasma glucose levels induced by clonidine, which was observed in males only, restrains the glucagon response. Thus a slight hyperglycemia in combination with the lower glucagon response in males but not in females was also observed after carbachol administration. It is, however, unlikely that the small increase in glycemia induced by either of the autonomic agonists would significantly contribute to the observed gender difference in the glucagon responses, because glucagon secretion usually does not change in the range of glucose concentration between 5 and 11 mM (25). The gender difference in the glucagon response to clonidine is therefore most likely centrally governed.

Previously, insulin was shown to suppress the glucagon response during insulin-induced hypoglycemia in both normal human subjects and type 1 diabetics (20, 21). However, such an action does not explain the observed gender difference in the glucagon response to 2-DG or insulin-induced hypoglycemia in this study, because plasma insulin levels increased to an equal extent in both genders. On the contrary, the insulin response to cholinergic agonism was lower in males, which would tend to increase (not reduce) glucagon secretion.

Our finding of a higher glucagon response to 2-DG and insulin-induced hypoglycemia in female mice is in contrast to studies in humans during insulin-induced hypoglycemia; in two studies, a decreased glucagon response was found in females, whereas no difference between genders was evident in one study (3, 9, 11). The mechanism underlying this difference between humans and mice is not known, but our results emphasize a fundamental species difference regarding the regulation of the glucagon response to glucopenic stress between humans and mice. Regarding the sympathoadrenal component of the counterregulatory response to glucopenia, we found plasma catecholamines to be higher in males during insulin-induced hypoglycemia, which is in agreement with previous studies in human subjects where a higher epinephrine response was observed in males during insulin-induced hypoglycemia (3, 9, 11). In contrast, during 2-DG-induced glucopenic stress, no gender differences in the plasma levels of epinephrine and norepinephrine were observed in the present study in mice, which indicates a gender-dependent difference in the counterregulatory response between glucopenic stress induced by insulin and that induced by 2-DG.

Although the present study was not designed to investigate any differences in basal plasma levels of insulin, glucagon, and glucose, we found in the 2-DG experiments that nonfasting plasma glucose levels were slightly higher in control males compared with control females. However, this was not the case when all 2-min control plasma glucose values were compared. This might be interpreted as if males have a tendency toward higher plasma glucose levels compared with females, and that a slight stress-related hyperglycemia occurs at 10 min compared with 2 min. This hypothesis is supported by the finding of higher plasma norepinephrine levels in males compared with females during control conditions. Previously, in studies on humans, females have been shown to have a lower capacity to maintain glycemia during fasting compared with males (9, 23), and in mice of the NMRI strain, fasting plasma glucose levels are lower in females compared with males (1). During control conditions, males exhibited higher plasma insulin concentrations, which indicates decreased insulin sensitivity in males compared with females. This confirms previous observations in the NMRI mouse (1, 5, 6). It might be speculated that the higher plasma insulin levels contribute to the lower plasma glucagon levels that are also observed in males, because insulin is known to inhibit glucagon secretion by a paracrine or endocrine mechanism within the islets (27). To what extent differences in sex hormones or other gender-dependent mechanisms contribute to the differences in the parameters of glucose homeostasis observed between genders in the present study remains to be established. However, our study emphasizes the existence of a gender-dependent regulation of carbohydrate metabolism in mice.

From the present study, we conclude that in the NMRI mouse, *1*) the glucagon response to glucopenic stress induced by 2-DG or insulin-induced hypoglycemia is markedly larger in female compared with male mice; *2*) the mechanism underlying the increased glucagon response does not rely on a different degree of sympathoadrenal activation; and *3*) the pancreatic α-cell of females has an increased sensitivity to cholinergic activation, which may contribute to the larger glucagon response observed in females during glucopenic stress.

Perspectives

The present study has clearly demonstrated that gender is an important determinant for the regulation of glucose homeostasis in mice, which was previously demonstrated for other species including humans. The present study, however, emphasizes that the influence of gender might differ between species. Thus the observation that the glucagon response to glucopenia is larger in female mice than in male mice contrasts to the results of previously published studies in humans, which report that glucagon responses to insulin-induced hypoglycemia are larger in men than in women. The present study demonstrates that an increased sensitivity of the α-cell to cholinergic activation might contribute to the larger glucagon response to glucopenic stress in mice. Because the pancreatic content of glucagon has previously been demonstrated to be increased in female versus male mice, the possibility exists that an increased α-cell mass also contributes to the enhanced glucagon response in females. The exact mechanisms underlying the gender differences in glucose homeostasis have not been established. Future studies, particularly those examining the influence of reproductive hormones, are warranted.

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